

Improved sapflow methodology reveals considerable night-time ozone uptake by Mediterranean species

S. Mereu¹, G. Gerosa², A. Finco², L. Fusaro¹, B. Muys³, and F. Manes¹

¹University of Rome “Sapienza”, Department of Plant Biology, P. le Aldo Moro 5, 00152, Rome, Italy

²Catholic University of the Sacred Heart, Department of Mathematics and Physics, Via Musei 41, 25121 Brescia, BS, Italy

³Katholieke Universiteit Leuven, Department Forest, Nature and Landscape, Celestijnenlaan 200E, 3001, Leuven, Belgium

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Abstract. Due to the evident tropospheric ozone impact on plant productivity, an accurate ozone risk assessment for the vegetation has become an issue. There is a growing evidence that ozone stomatal uptake may also take place at night and that the night-time uptake may be more damaging than diurnal uptake. Estimation of night-time uptake in the field is complicated because of instrumental difficulties. Eddy covariance technology is not always reliable because of the low turbulence at night. Leaf level porometry is defective at relative humidity above 70% which often takes place at night. Improved sap flow technology allows to estimate also slow flows that usually take place at night and hence may be, at present, the most trustworthy technology to measure night-time transpiration and hence to derive canopy stomatal conductance and ozone uptake at night. Based on micrometeorological data and the sap flow of three Mediterranean woody species, the night-time ozone uptake of these species was evaluated during a summer season as drought increased. Night-time ozone uptake was from 10% to 18% of the total daily uptake when plants were exposed to a weak drought, but increased up to 24% as the drought became more pronounced. The percentage increase is due to a stronger reduction of diurnal stomatal conductance than night-time stomatal conductance.

(Musselmann et al., 2006; Karlsson et al., 2007), what is effectively relevant for plants is the ozone stomatal uptake rather than the exposure (Uddling et al., 2004). This implies that ozone risk assessment requires a precise knowledge of canopy stomatal conductance (G_s , m s^{-1}).

The eddy covariance technique (EC) is recognized to be the most direct method to measure ecosystem-scale fluxes of H_2O , CO_2 , and other gases (Swinbank, 1951; Hicks and Matt, 1988; Grunhage et al., 2000). EC, by the aid of some assumptions, allows to evaluate canopy conductance (G_c , m s^{-1}) from the EC dataset by inverting the Penman-Monteith equation (Monteith, 1981) or by using a simple electric analogy (Gerosa et al., 2007). Although the EC method is a powerful tool, eventual gas exchange occurring at night remain “unseen” mainly because of the low atmospheric turbulence (Fisher et al., 2007). Because of this, sap flow technology may be a valid alternative to EC for night-time evaluation of gas exchange (Fisher et al., 2007; Grulke et al., 2004).

Even though different authors have detected either night-time transpiration (E_n) or night-time stomatal conductance ($g_{s,n}$) (Muchow et al., 1980; Furukawa et al., 1990; Wieser and Havranek, 1995; Anderson, 1982; Rawson and Clarke, 1988; Bakker, 1991; Daley and Phillips, 2006; Green et al., 1989) the idea that stomata remain closed at night is still the most diffused. For an accurate review refer to Caird et al. (2007). Dawson et al. (2007) demonstrate that E_n takes place over a great variety of plant species using datasets from 10 study sites representing six biomes on three continents, plus the Hawaiian Islands. Their work was possible thanks to the advancements in sap flow measurements which allow to measure also very low sap flow rates. They report values of stomatal conductance (g_s) as high as 20% of the daily

1 Introduction

Ozone (O_3) is recognized to be a cause of significant losses of plant Primary Productivity and, as discussed elsewhere



Correspondence to: S. Mereu
(simone.mereu@uniss.it)

maximum, while Caird et al. (2007) report values as high as 50% of the daily maximum. Even if the occurrence of E_n is a recognized process, a physiological explanation of E_n is still lacking and to date it is not possible to determine whether E_n represents a passive process, possibly due to stomatal leaking, or it has an adaptive meaning. The difficulty in explaining the physiological significance arises from the apparently contradictory differences in the response of E_n and $g_{s,n}$ to environmental conditions that has been observed between species. Howard and Donovan (2007), for example, demonstrate that the reason for E_n is not in a higher nutrient uptake and it does not depend on leaf age in nine *Helianthus* species, but Scholz et al. (2007) and also Ludwig et al. (2006) find an inverse correlation between nutrient availability and E_n . The correlation between $G_{s,n}$ and Vapour Pressure Deficit (VPD; kPa) is also not clear as, Dawson et al. (2007) found a positive correlation between them, while Cavender-Bares et al. (2007) found a negative correlation. Worth of notice is the different behaviour of shrub understorey species and of dominant trees reported by Fisher et al. (2007) in the same Mediterranean type ecosystem. In that article it is reported that E_n of the dominant species increases in summer, higher VPD and lower Soil Water Content (SWC; v/v %), while that of the understorey increases in winter, higher SWC and lower VPD. Even if the authors did not estimate $g_{s,n}$, it can be inferred from their data that the $g_{s,n}$ of the shrub species was higher in winter, in fact for transpiration to be greater at a lower VPD a higher $g_{s,n}$ is necessary. The surprising increase in $g_{s,n}$ with VPD is in strong contradiction with a water saving strategy because it means that stomata allow the plant to spend more water when the potential transpiration is higher. This response is also in contrast with the known day-time physiology, where G_s declines with VPD. These species-specific differences suggest a genetic control and hence the possibility for E_n to be an adaptive strategy (Rosenthal et al., 2002; Brouillette et al., 2006), that must still be understood.

Although the night-time physiology of G_s is still not understood, it has a relevant implication on the water balance of ecosystems. Howard et al. (2007), Dawson et al. (2007) and Bucci et al. (2005) quantify E_n to be between 5% and 20% of daily maximum transpiration.

The occurrence of night-time stomata opening could also be relevant for pollutant uptake by plants (Grulke et al., 2004; Matyssek et al., 1995). Stomatal represent in fact the passage way for ozone to enter the leaf lamina. In Mediterranean ecosystems ozone damage may be partially avoided because high concentrations of O_3 during day light co-occur with drought, which brings to stomatal closure (Gerosa et al., 2009; Manes et al., 2005). Since night-time G_s may be significantly different from 0, it is plausible that ozone stomatal uptake takes place at night. Ozone night-time may represent a significant fraction of the total ozone uptake since night-time ozone concentrations in Italy vary between 10 and 45 ppb across both rural and suburban areas (Paoletti, 2006).

Not considering that night-time ozone uptake may cause a greater damage at an equivalent dose as at night there is no or little scavenging antioxidant made by photosynthesis (Loreto and Fares, 2007).

However, the estimation of night-time stomatal uptake must face both physiological (Barbour and Buckley, 2007) and technical difficulties (Aubinet, 2008). The physiological problem is mostly due the poor knowledge of how plants control stomata opening at night and to which environmental and physiological conditions they respond to. The technical problem, instead, consists in the difficulties to measure fluxes and in estimating $g_{s,n}$ in an environmental situation typically characterized by low turbulence and high humidity. These difficulties imply that, even if ozone night-time uptake may be relevant for the Mediterranean ecosystems and forests in general, the estimation of the dose requires both knowledge of the species specific response to environmental parameters and of an appropriate instrumental approach.

In this work, we first analyse the species night-time physiology of three Mediterranean species (*Arbutus unedo* L., *Quercus ilex* L. and *Erica arborea* L.). Finally, we estimate night-time ozone fluxes, evaluate their significance for the ozone risk assessment and discuss the opportunity to couple EC with sap flow technology.

2 Material and methods

2.1 Study site

The study site is a Mediterranean maquis dominated by *Q. ilex*, but the limiting environment due to a poor soil and vicinity of the sea determines the presence of typical species of the garrigue (*Erica multiflora*, *cistus* sp. and *Rosmarinus officinalis*). The climate is typically Mediterranean with a pronounced summer drought and rain events concentrated in autumn and spring, with a mean annual total precipitation of 780 mm per year. Mean monthly temperatures range between a minimum of 6 °C and a maximum of 24 °C while the relative humidity (RH) is rarely below 50%. For a more detailed description refer to Fares et al. (2009).

2.2 Environmental monitoring

Three thermo-hygrometer probes (50Y, Campbell, USA) were placed along a vertical axis at 0.1, 1 and 3.8 m height. Two leaf wetness sensor (237, Campbell, USA) were placed in open space above two 1 m high shrubs at 1.5 m height. Three thermocouples (PT100, Delta T, UK) with 0.1 °C accuracy were placed next to the main vein on the abaxial page of three mature *Q. ilex* leaves pointing east, south and west respectively in order to measure leaf temperature (T_l). The three PT100 were inserted inside the radiation shield of the thermo-hygrometers for calibration before and after the measuring period. The relationship between the two sensors was

found linear ($m = 0.98$; $b = 0.23$). All sensors were connected to a CR10x data logger (Campbell, USA) which acquired every 15 s and stored them as 30 min averages. Additional information about the sensors can be found in Gerosa et al. (2009) since the same sensors were used.

2.3 Sap flow measurements

Sap flow measurements were performed on *Q. ilex* ($n = 4$) and on *A. unedo* ($n = 4$) and are the same described in Mereu et al. (2009). For this study, an additional species (*Erica arborea* L., $n = 3$) was monitored. The sap flow sensors are based on the Heat Field Deformation system (HFD) (Nadezhdina et al., 2004). The method is part of a family of sensors based on the heat transfer methods, in this case two pairs of thermocouples measure changes in the temperature difference (ΔT) caused by the deformation of the heat field around the heater due to the moving of sap. The so-called symmetric thermocouple measures the difference in temperature between two equidistant points above and below the heater, while the asymmetric thermocouple measures the temperature between the lower point and another point set at 0.5 cm abreast the heater. This system has been shown to be adequate for both high and low sap flow rates (Nadezhdina et al., 2008).

In an analytically form, it can be found that sap flow density, Q , can be calculated as

$$Q = \frac{3600 \cdot D \cdot (dT_{\text{sym}}/dT_{\text{as}}) \cdot Z_{\text{sym}}}{L_{\text{sw}} \cdot Z_{\text{tg}}} \quad (1)$$

or

$$Q = \frac{3600 \cdot D_{\text{st}} \cdot (dT_{\text{sym}}/dT_{\text{as}}) \cdot Z_{\text{sym}}}{A} \quad (2)$$

where 3600 transforms seconds into hours; D is the thermal diffusivity of green (fresh) wood ($\text{cm}^2 \text{s}^{-1}$); dT_{sym} and dT_{as} are the temperature differences ($^\circ\text{C}$), recorded by symmetrical and asymmetrical thermocouples, respectively; Z_{sym} is the axial distance between the symmetrical pair of thermocouples (cm); Z_{tg} is the tangential distance between the heater and the upper end of the asymmetrical pair of thermocouples (cm); A is the conducting measured area, where deformation of heat field is studied. This area is limited by the heater and the upper end of asymmetrical thermocouples and depth of sapwood, L_{sw} :

$$A = L_{\text{sw}} Z_{\text{tg}} \quad (3)$$

where L_{sw} can be determined from measurements of the sap flow radial pattern by radial profile sensors.

The sensors were insulated from the surrounding environment by coating them with silicon and wrapping them in aluminum cover.

All sensors were connected to a DL2 data logger (Delta-T devices, UK) which acquired data from the sensors every minute and stored them as 30 minutes averages. In three different days data was acquired and stored at 1 min intervals for

24 h to estimate the time lag between environmental drivers (Radiation and VPD) and sap flow.

2.4 Stomatal conductance

Leaf stomatal conductance measurements at night were not collected with a porometer because RH was most often above 80% and both steady state or dynamic diffusion porometers are not reliable at relative humidity values above 70% (McDermitt, 1990).

Sap flow density (Q_1 ; $\text{Kg m}^{-2} \text{s}^{-1}$), measured in a known sapwood area (SA) can also be expressed per unit of Leaf Area (LA) after multiplication by the sapwood/leaf area ratio (LA/SA). For such a derivation, we used the LA/SA ratio of the species collected in Mereu et al., 2009. Using the transpiration per unit leaf area, G_s can be derived from sap flow measurements, based on a simplification of the Penman-Monteith equation (Whitehead and Jarvis, 1981; Pataki et al., 1998; Martinez-Vilalta et al., 2003; Kurpius and Goldstein, 2003):

$$G_s = \frac{\gamma \cdot \lambda \cdot Q_1}{\rho \cdot c_p \cdot \text{VPD}} \quad (4)$$

where γ is the psychrometric constant (kPa K^{-1}), λ is the latent heat of vaporization of water (J kg^{-1}), ρ is the density of air (kg m^{-3}), c_p is the specific heat of air at constant pressure ($\text{J kg}^{-1} \text{K}^{-1}$) and VPD is the vapour pressure deficit (kPa) of the air. The simplification can be considered valid if G_s is predominant over the leaf boundary layer conductance, g_b (Whitehead and Jarvis, 1981) i.e. when the canopy is strongly coupled with the atmosphere. The Mediterranean vegetation is usually assumed to be well coupled with the atmosphere (Martinez-Vilalta et al., 2003; Infante et al., 1997) given its general small leaf size (Gratani and Varone, 2004; Gratani and Bombelli, 2000). This assumption is not necessarily valid at night when RH may be high and wind intensity is usually low, two conditions that are known to cause the decoupling of the vegetation (Monteith and Unsworth, 1990). To test the degree of coupling with the atmosphere, the value of g_b was calculated for all three species and compared with G_s using the equation proposed by Jones (1992):

$$g_b = 6.62 \cdot \left(\frac{u}{d}\right)^{0.5} \quad (5)$$

where d is the average leaf size and u is the wind speed. The samples that were not well coupled were determined by means of an iterative procedure where samples with increasing g_b values were excluded at each step until the correlation coefficient between E_n and VPD did not increase of more than one hundredth.

2.5 Eddy covariance flux measurements

The micrometeorological instrumentation was mounted over a scaffold at 3.8 m from the ground. The three components

of the wind vector and the temperature were measured at high sampling frequency (20 Hz) by means of a ultrasonic anemometer (mod. USA-1, Metek, Germany). Air moisture was recorded with a fast-response sensor (mod. 7500, LiCor, USA). Half hourly ozone concentration means were measured with an automatic analyzer (S-5014, SIR, E) based on UV absorption.

Starting from the fast anemometer data, the friction velocity u^* , a good descriptor of the atmospheric turbulence, was calculated as:

$$u^* = \sqrt[4]{(w'v')^2 + (w'u')^2} \quad (6)$$

where u' and v' are the wind speed fluctuations along the x and y axis respectively and w' is the vertical wind speed.

Latent heat was measured by using the micrometeorological approach, assuming that fluxes are independent from height in the lowest air layers (Stull, 1988). Hence, a flux measured at several meters above ground level is assumed to be equal to the flux at the air – vegetation interface. The flux was determined by the eddy covariance method, which is considered to be the most reliable (Hicks and Matt, 1988). Fluxes are proportional to the covariance between turbulent fluctuations of the vertical wind vector component and of the scalar entity of interest: water vapour for latent heat fluxes, and air temperature for sensible heat fluxes. Calculation of the fluctuations requires averaging over successive periods of time, in order to eliminate non-turbulent, long-term variations. The averaging time was chosen such as to include all turbulent fluctuations (at least 10 min) occurring in the atmospheric surface layer, but it had to be short enough to avoid the synoptic-scale fluctuations (less than 1 h, Van der Hoven, 1957). A 30-min averaging time was taken as a compromise. Ozone concentrations at the height of interest (1.5 m, around the average height of the three studied species) were not available, hence they were estimated by the gradient method taking the “turbulent diffusion” coefficient for heat, K_H , directly derived from the EC measurements:

$$C_h = C_{z_m} + \frac{F}{K_H} \cdot (z_m - h) \quad (7)$$

with

$$K_H = k \cdot u^* \cdot (z - d) / \Phi_H(\zeta) \quad (8)$$

and the similarity function $\Phi_H(\zeta)$ accounting for the atmospheric stability (Dyer, 1974) being

$$\phi_H = \begin{cases} [1 - 16 \cdot \zeta]^{-1/2} & \text{for } \zeta < 0 \text{ unstable conditions} \\ [1 - 5 \cdot \zeta]^{-1} & \text{for } \zeta \geq 0 \text{ stable conditions} \end{cases} \quad (9)$$

with $\zeta = (z - d)/L$ L is the Monin-Obukhov length, obtained from u^* and the sensible heat flux measurements (Stull, 1988), C_h is the ozone concentration at 1.5 m, C_{z_m} is the ozone concentration at the measurement height $z_m=3.8$ m, h is the canopy height equal to 1.5 m and F is the ozone flux (ppb m s⁻¹).

2.6 Ozone fluxes

Ozone fluxes were calculated using:

$$FO_{3,\text{stom}}(i) = [O_3(i)] \cdot G_s(i) \cdot 0.613 \quad (10)$$

where $FO_{3,\text{stom}}$ is ozone stomatal flux (nmol m⁻² s⁻¹), $[O_3]$ is ozone concentration (nmol m⁻³), G_s is stomatal conductance (mm s⁻¹) and 0.613 is the ratio between the diffusion coefficient of O₃ and that of water vapour (Massman, 1998). Ozone concentrations were measured with a fast-response chemiluminescence ozone sensor (OS-G-2, GFAS) and a slow-response ozone analyzer (mod. 400, Advanced Pollution Instrument) as reference (Gerosa et al., 2009). Ozone stomatal fluxes were calculated only when the leaves could be assumed coupled with the atmosphere using the procedure described above.

3 Results and discussion

3.1 Environmental monitoring

During the measuring period only two precipitation events occurred in the first half of the campaign, totalling 14 mm of rain. VPD at 3.8 m height varied between 0.1 and 2.9 kPa and air temperatures T_a ranged between 10 and 32 °C, both parameters had higher values as the season progressed. SWC at 100 cm depth declined constantly from 13% to 5.8% during the campaign, however the flow of the studied species was kept relatively high thanks to the presence of a water table (Mereu et al., 2009).

Mean T_a and mean VPD during the night (21:30–06:00) at 1 m height were 14.46 °C and 0.2 kPa. Typically, leaf wetness increased from 19:00 and reached complete wetness around midnight, while leaves dried from 06:30 to 09:30 (Fig. 1a). The slope of the vertical gradients of VPD and T_a were negative during the day and positive at night, typically inverting after sunset (19:00 p.m.) and after dawn (07:00 a.m.), while the vertical gradient of RH showed the opposite change in gradients, from positive in the day to negative at night (Fig. 1a, b, c).

Ozone concentrations at 3.8 m height were comprised between 0 and 108 ppb with an average of 35 ppb (Gerosa et al., 2009), while ozone estimated at 1.5 m height was between 0 and 87 ppb. At night, ozone concentrations were on average between 30 and 11 ppb, with the higher concentrations occurring in the first hours after sunset (Fig. 2a). The mean night-time ozone concentration at 1.5 m was 23 ppb, but higher concentrations occurred when the wind blew from the sea (Fig. 2b).

3.2 Water fluxes

The experimental period was characterized by two periods (15 May–12 June and 13 June–31 July) with a different water availability that caused a reduction in the Latent Heat (LE)

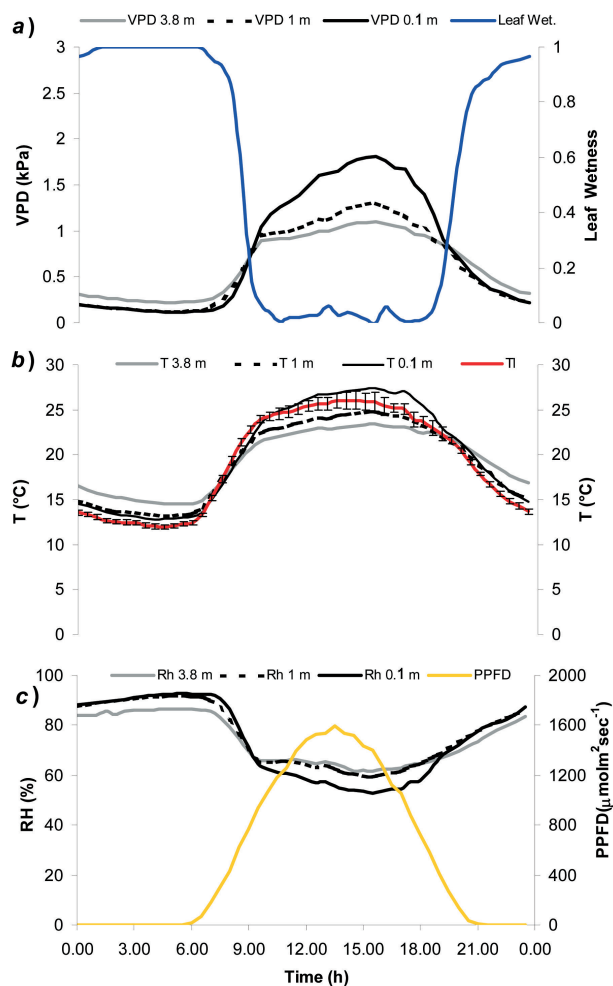


Fig. 1. Average of the half-hourly measurements of environmental parameters calculated using data from the entire experimental campaign (from 15 May to 31 July, 2007). **(a)** Vapour Pressure Difference (VPD, kPa) at tree different heights (3.8 m; 1 m; 0.1 m), and frequency of canopy wetness (ratio of the number of half an hours with wet canopy to the total number of half an hours) in the different hours. **(b)** Air temperature T_a (°C – 3.8 m; 1 m; 0.1 m) and leaf temperature T_l (°C) of *Q. ilex*. **(c)** Relative Humidity (RH, %) and solar radiation (PPFD, $\mu\text{mol m}^{-2} \text{s}^{-1}$).

of the ecosystem (Fig. 3a, b) (Gerosa et al., 2009). In both periods sap flow rose during the day (Fig. 3a, b) following the trend of the net radiation and VPD (Fig. 1c), the two driving forces of LE (Monteith, 1965). The effect of the different water availability was reflected mostly by a diurnal decrease of sap flow, with *A. unedo* being the species that underwent the higher relative reduction (Fig. 3a, b). Night-time sap flow instead did not vary greatly between the two periods.

Night-time values of VPD, the only potential driver of transpiration in the absence of radiation, was greater than zero at all heights (Fig. 1a), hence allowing for a potential transpiration.

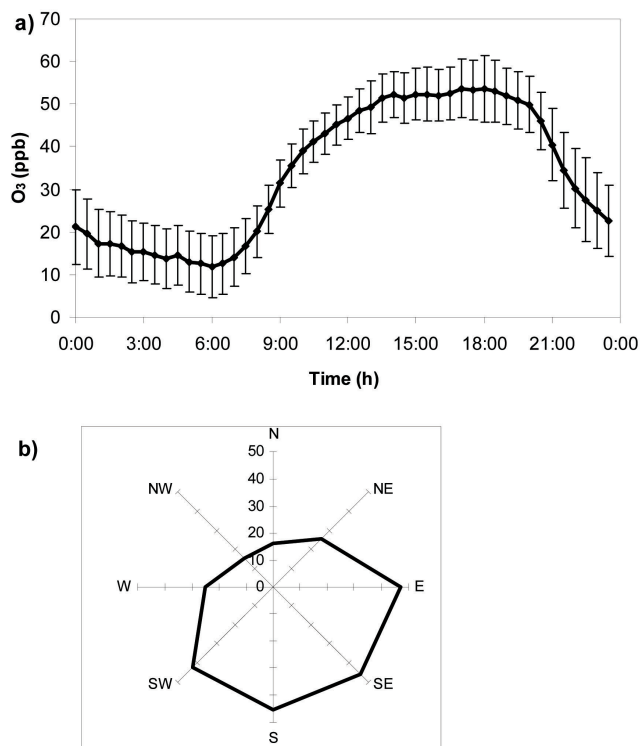


Fig. 2. **(a)** Average of the half-hourly measurements of ozone concentrations at 1.5 m height. **(b)** Night-time ozone concentration as function of wind direction. When the wind is blowing from the sea (SW-S direction), concentrations are higher.

A positive sap flow (from roots to leaves) was recorded by the sensors also at nights when EC measurements could not be considered reliable because of the low turbulence and canopy wetness (Gerosa et al., 2009). Sap flow represents an indirect measurement of transpiration and its reliability must be judged with attention. The reliability of the measurement was strengthened by the constantly lower temperature of the lower end of the symmetrical pair of thermocouples (cf. Sect. 2.3), meaning that heat was transferred upwards from the heater to the upper end of the thermocouple. The occurrence of transpiration at night is also confirmed by the leaf temperature T_l of *Q. ilex*: T_l was higher than T_a at 1 m height during the day because of the incoming solar radiation, but lower at night, typically inverting at 07:30 and 19:30 (Fig. 1b). It should be considered that T_l during the day may vary greatly inside the canopy since the radiation intercepted by each leaf depends on its position inside the canopy, at night instead the difference between the T_l of different leaves is reduced. Between 23:30 and 05:00, T_l was almost constantly 1.26 °C lower than T_a . This difference can be sustained only if heat is continuously extracted from the leaf, i.e. transpiration occurs at the expenses of the internal heat of the leaves and not at the expenses of the surrounding air. A further evidence is that when wind intensity rises, the difference between T_l and T_a (ΔT) tends to 0 with a logarithmic

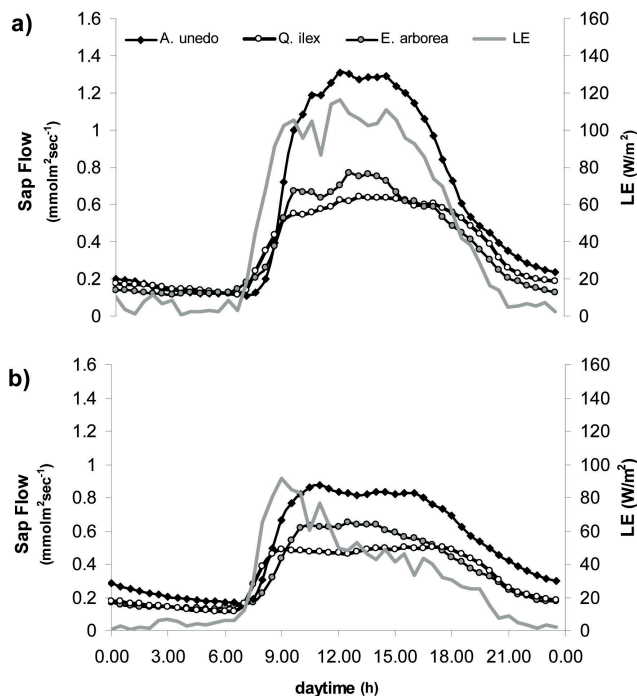


Fig. 3. Average of the half-hourly measurements of sap flow rates of tree species and total ecosystem LE from Eddy Covariance. Values shown are for the mean day of (a) first period and (b) second period. Modified from Gerosa et al., 2009.

relationship (Fig. 4a). High wind intensities, in fact, lead to a thinning of the leaves boundary layer (increased g_b , cf. Eq. 5) to which *Q. ilex* and *A. unedo* plants respond with a reduction of $g_{s,n}$ (Fig. 4b). The reduction of g_b also favours an exchange of heat from the warmer air to the colder leaves that together with the reduction of $g_{s,n}$, favours the thermal re-equilibration of the canopy with the surrounding air.

Caution was also given in differentiating night-time refilling from actual water loss. Refilling occurs during the night in order for the plant to re-equilibrate the leaf water potential with that of the soil, hence for a determined period of time the leaf water potential (Ψ_l) tends to equal the soil water potential (Ψ_s). The time necessary for this process largely depends on the amount of water that must be replenished in the plant tissues at the end of the day and on the capacitance of the plant, which in turn depends on plant volume, wood elasticity and wood density. In this study, plants were rather small in size (Fares et al., 2009) and had a high wood density as most Mediterranean woody species (Cherubini et al., 2003), in accordance with their adaptation with a dry environment (Hacke et al., 2001). The influence of refilling on the sensors also depends on the distance that separates the sensor from the transpiring surfaces and an additional reduction of the influence of refilling on transpiration was achieved by placing the sensors at less than one meter from the crown.

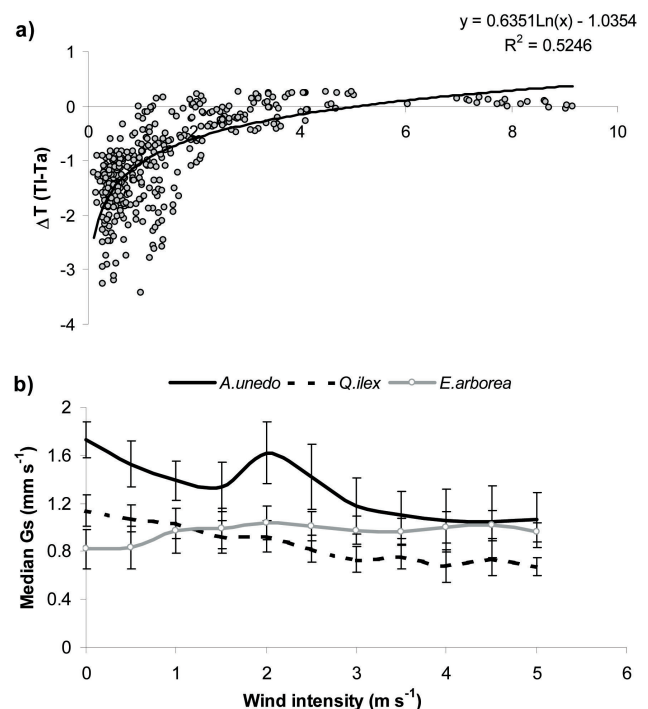


Fig. 4. (a) The ΔT between air and leaves of *Q. ilex* diminishes logarithmically with wind intensity (m s⁻¹). (b) Median of canopy conductance, G_s (mm s⁻¹) as function of wind intensity (m s⁻¹) for the tree species (*A. unedo*, *Q. ilex*, *E. arborea*) showing how stomata tend to limit transpiration. Maximum wind speed in the two panels does not coincide because sap flow measurements were not available (instrumental break down) in the only day when wind speed exceeded 5 m s⁻¹.

A time lag between sap flow at stem level and sap flow at branch level or between sap flow and environmental variables is often considered as a proxy of the capacitance of the plant tissues (Phillips et al., 2008), where capacitance is the change in tissue water content per unit change in water potential. However, Burgess and Dawson (2008) observed that the time lag is not always clearly proportional to the capacitance and that this variability may be explained by other water movement inside the tissues or instrumental artefacts. In fact, sap flow based on continuous heating, such as in this study, may bring to a thermal inertia where the increase or decrease in sap flow is not immediately sufficient to determine changes in the temperature gradients around the heater. The fast recording sub-sampling periods allowed to determine a time lag of about eight minutes between sapflow and radiation or VPD (data not shown) which is well below the 30 min average used for the study and hence does not effect the interpretation of the data. Such a short time lag also suggests a reasonable accuracy of the HFD sensors and confirms the expected low capacitance of the three species in agreement with their small size and high wood density.

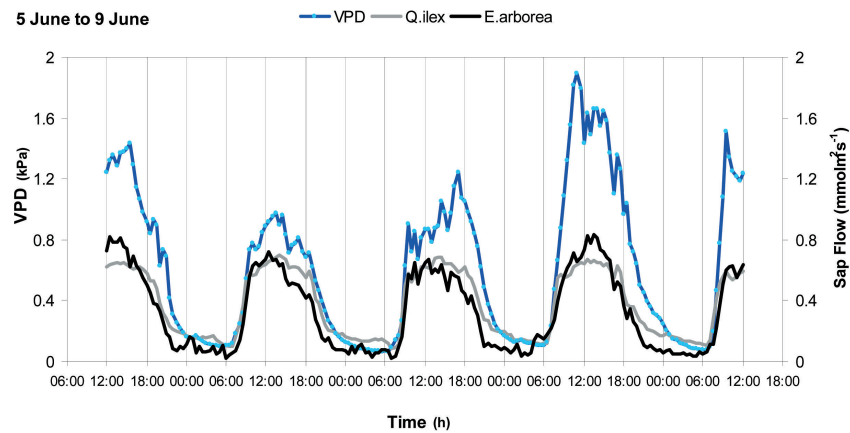


Fig. 5. Daily course of VPD and sap flow of *Q. ilex*, and *E. arborea* from 5 June to 9 June 2007.

Even if in this study the effect of capacitance on sap flow measurements is minimized, a refilling process is assumed to be taking place after sunset and for a period of time the amount of water entering the plant is higher than the amount transpired. It has often been observed that sap flow declines after sunset and tails off to a quasi plateau in a period of time dependent on the species. Such a behaviour is recognizable in the mean days presented in Fig. 3 and in the sample days for *Q. ilex* and *E. arborea* (Fig. 5) and persist for a period of about three hours after sunset. The period between sunset and the achievement of the quasi plateau can be considered a period where transpiration occurs together with refilling (Fisher et al., 2007) and where it is complicated to estimate the relative proportion of the two processes. The following period, instead, can be considered in a steady state equilibrium where the amount of water entering the plant equals the amount transpired. Difficulties in differentiating refilling from transpiration can cause inaccuracies in the estimation of transpiration at night which can be quantitatively relevant especially for big trees. In order to avoid a potential over-estimation during this period, transpiration in the first three hours after sunset was assumed to be equal to the average night transpiration. However, this is likely to underestimate the night-time contribution to transpiration as VPD in the first three hours after sunset was generally higher than in the rest of the night.

Even if the occurrence of transpiration at night has been reported for several species in a wide range of environmental conditions (Caird et al., 2007) either with sap flow gauges, leaf level measurements of gas exchange or EC, night-time transpiration remains a controversial topic as it is a complicated measurement and because of its implication for plant physiology and ecosystem gas exchange measurements. In fact, in the presence of high humidity and low turbulence, as in this study at night, LE measurements with EC are largely impaired and, as expected, EC was not able to deliver reliable LE measurements during the night: only 156 cases out of 1593 satisfied the imposed stationarity and dry canopy con-

ditions (Gerosa et al., 2009) between 21:00 and 06:00. Even when the conditions were satisfied, caution should be used in considering the measured LE as true evapo-transpiration: in presence of a stable boundary layer, intermittent turbulence produces strongly non stationary events during which the validity of turbulent transport is uncertain (Aubinet, 2008). In agreement with Fisher et al. (2007), sap flow measurements appear to be more reliable than EC in these conditions and at night in general. However, besides the technological complications discussed above, low humidity and low turbulence, represent a complication also for the estimation of transpiration from sap flow technology. In fact, in these conditions it is necessary to establish the degree of coupling of the vegetation with the atmosphere. In these conditions, that characterized our site at night, the measured LE peaks may be the result of the release of water accumulated in the precedent hours in the atmospheric strata below 3.8 m, from the sensors to the ground. This is supported by the fact that the few EC measured water fluxes were, at least, one order of magnitude greater than the transpiration measured with the sap flows.

E_n occurred throughout the season in all three species (Fig. 3) and had a positive correlation with VPD (Fig. 6) in agreement with other authors (Dawson et al., 2007; Howard and Donovan, 2007; Fisher et al., 2007). A significant improvement of the correlation was achieved by discarding data for low values of g_b with the procedure described in Sect. 2.4. Species differed in the g_b value below which the vegetation was considered partially decoupled from the atmosphere: a value of 17 and 21 mm s^{-1} was found for *A. unedo* and *Q. ilex* while a value of 32 mm s^{-1} was found for *E. arborea*. While the values for the first two species correspond to the same wind intensity (0.41 m s^{-1}), the value found for *E. arborea* corresponds to a lower wind speed of 0.33 m s^{-1} . The percentage of data that was discarded varied between species both because of the different coupling and because of the different number of measurements available for each species. More precisely, 112 measurements out of 1058 for *A. unedo*,

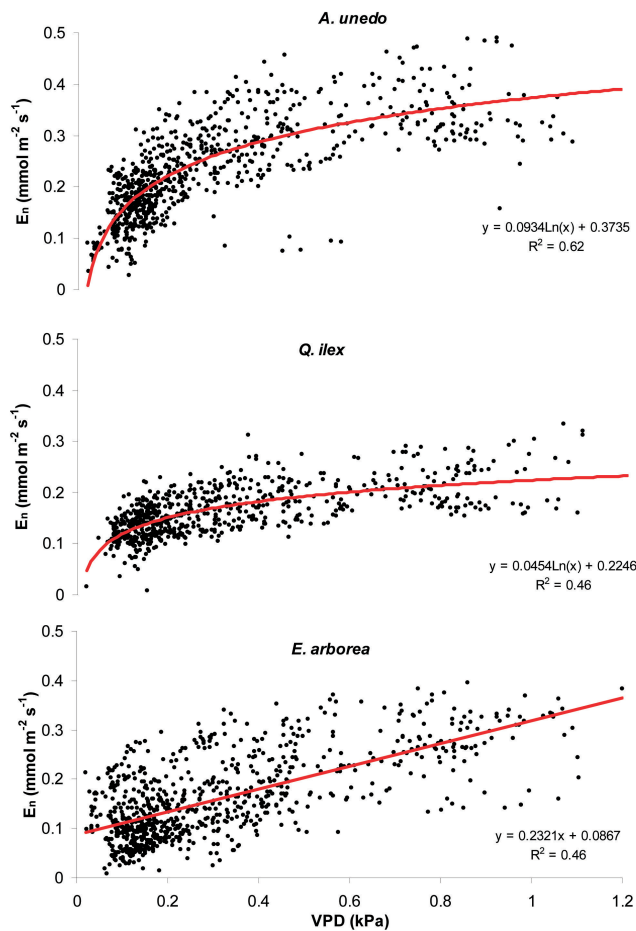


Fig. 6. Relationship between canopy transpiration at night (E_n) and mean vapour pressure deficit (VPD) for each studied species. Best curve fit was: logarithmic for *A. unedo* and *Q. ilex* and linear for *E. arborea*.

104 out of 942 for *Q. ilex*, and 58 out of 1158 for *E. arborea* were discarded from the further data processing. The procedure allowed for an appreciable increase in the R^2 of the best curve fit: from 0.49 to 0.62 for *A. unedo*, from 0.39 to 0.46 for *Q. ilex*, and from 0.40 to 0.46 for *E. arborea*. The R^2 of these correlations is slightly lower than that reported by other authors, but it must be considered that in our experimental period VPD varied only between 0.2 and 1 kPa while Fisher et al. (2007) report mean night VPD values in a range from 0 to 2.5 kPa, a range that allows for a better correlation to be achieved. Also differently from Fisher et al. (2007), our best fit consisted in a logarithmic and not in a linear relationship for *A. unedo* and *Q. ilex* (the R^2 of the linear fit was 0.56 and 0.42 for *A. unedo* and *Q. ilex* respectively). Given the improvement achieved the R^2 of these relationships, we strongly recommend such or similar tests before further processing sap flow datasets.

Doubts may rise on the reliability of $g_{s,n}$ through Eq. (4), since the equation does not include an energy balance as

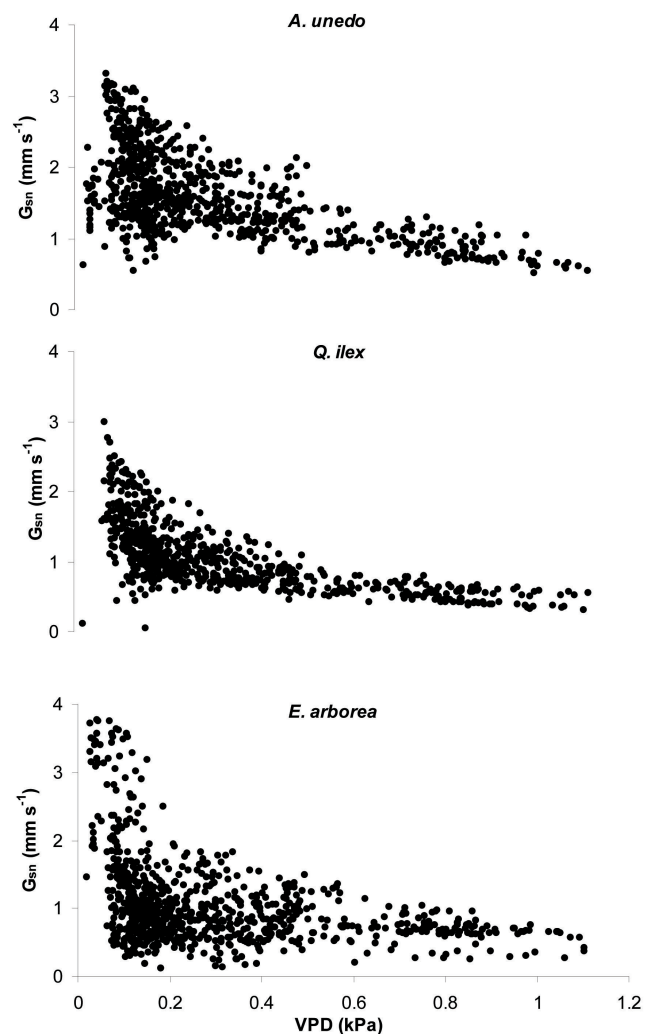


Fig. 7. Relationship between canopy stomatal conductance ($G_{s,n}$) and vapour pressure deficit (VPD) for each species using data collected at night (23:00 p.m. to 06:00 a.m.).

the original Penman-Monteith equation, nor it includes leaf temperature as in the Evaporation-Resistance approach proposed by Thom (1975). $G_{s,n}$ was indeed sensible to VPD values, but it must be considered that if the leaf temperature is lower than that of air, as it is at night, the resultant difference in vapour pressure between leaf and air (VPD_l) is lower than the atmospheric VPD. Hence Eq. (4) is likely to underestimate G_s at night when leaf temperature is lower than T_a ($VPD_l < VPD$) and overestimate it during the day when T_l is higher than T_a ($VPD_l > VPD$).

$g_{s,n}$ had a negative correlation with VPD for all three species (Fig. 7) and did not change relevantly between the first period (higher water availability) and the drier second period (Fig. 8), bringing to a weak correlation between soil water content and $G_{s,n}$ (data not shown).

In this study, $g_{s,n}$ of all three species responds to wind (Fig. 4b) and VPD (Fig. 7) indicating a water saving strategy

Table 1. Average daily ozone dose ($\text{nmol m}^{-2} \text{s}^{-1}$) for the three species in the first and in the second period and using ozone concentrations at two heights. Night fraction corresponds to the fraction of the total daily ozone uptake attributable to night fluxes only.

3.8 m	15 May to 12 June			13 June to 31 July		
	<i>A. unedo</i>	<i>Q. ilex</i>	<i>E. arborea</i>	<i>A. unedo</i>	<i>Q. ilex</i>	<i>E. arborea</i>
Diurnal flux	123.3	68.8	80.8	75.1	45.3	52.5
Night flux	20.4	12.0	20.5	28.2	19.0	18.7
Total flux	143.7	80.9	101.3	103.3	64.3	71.3
Night Fraction	0.14	0.14	0.20	0.27	0.29	0.26

1.5 m	15 May to 12 June			13 June to 31 July		
	<i>A. unedo</i>	<i>Q. ilex</i>	<i>E. arborea</i>	<i>A. unedo</i>	<i>Q. ilex</i>	<i>E. arborea</i>
Diurnal flux	120.3	66.5	78.6	74.1	45.1	50.8
Night flux	16.8	7.5	17.5	20.8	14.2	14.7
Total flux	137.1	74.0	96.1	94.9	59.2	65.4
Night Fraction	0.12	0.10	0.18	0.22	0.24	0.22

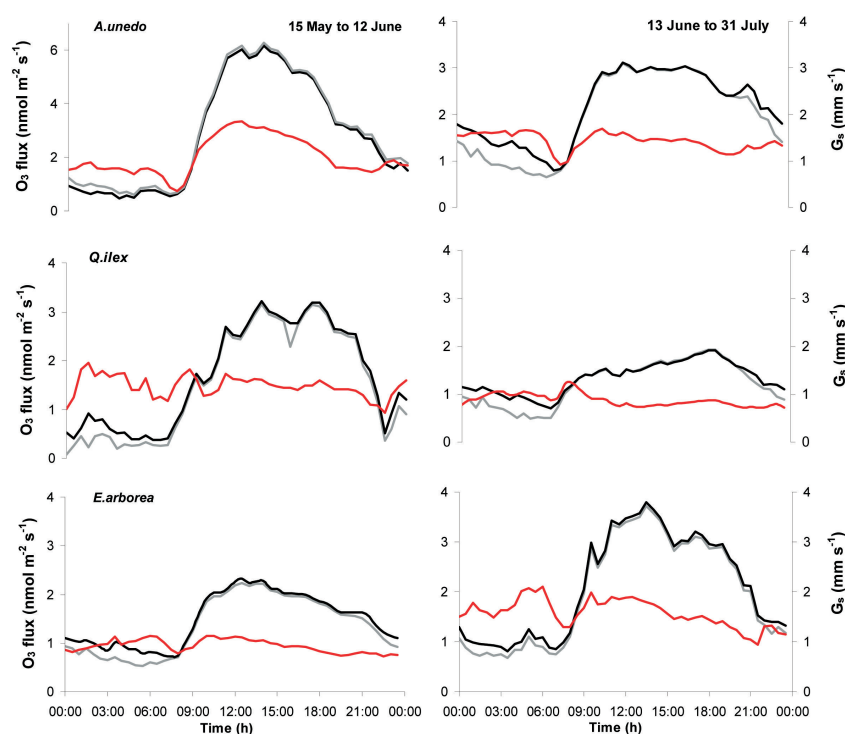


Fig. 8. Average of the half-hourly measurements of G_s (red line) and ozone stomatal fluxes of the three species in the first (left panels) and in the second period (right panels). Ozone fluxes were calculated using concentrations at two different heights. Grey lines show trend using ozone concentrations at 3.8 m and black lines at 1.5 m. Night-time flows slightly increase between the two periods for *Q. ilex* and *A. unedo*, while the day-time fluxes decrease for all three species.

in accordance with their daytime physiology and with their adaptation to the dry Mediterranean climate. However, given the high variability of responses to environmental parameters reported in the literature, such a conclusion cannot be extrapolated to other Mediterranean species or to the same species in different growing conditions. This response also suggests that E_n is not a passive process as considered by

Fisher et al. (2007), which would imply a linear relationship between E_n and VPD and a constant $g_{s,n}$. The physiological control of $G_{s,n}$ is also testified by the variation of $g_{s,n}$ during in the hours precedent to dawn (Bucci et al., 2004; Dodd et al., 2005), a variation that is also evident in Fig. 8, where $g_{s,n}$ diminishes before day light.

3.3 Ozone fluxes

Besides the importance to the water budget of the species, significant values of $g_{s,n}$ may imply important ozone stomatal uptake during the night. Despite the generally low ozone concentrations of the site, but notice that in some nights they were above 30 ppb, a significant fraction of the total (day and night) ozone uptake occurred at night (Table 1).

On average, night-time ozone fluxes remained similar in the first and in the second period (Fig. 8) and were on average below $1 \text{ nmol m}^{-2} \text{ s}^{-1}$, except for *A. unedo* in the second period where values of $1.5 \text{ nmol m}^{-2} \text{ s}^{-1}$ were reached around midnight. The small difference between the two periods is a consequence of similar night-time ozone concentrations and similar $g_{s,n}$ values across the experimental period. Using the concentrations at the two different levels, determines a substantial difference in ozone uptake only at night with an overestimation for the concentrations at 3.8 m. The $g_{s,n}$ response to the increasing VPD and aridity during the experimental period is weaker than that of diurnal G_s (Mereu et al., 2009), and this determines a higher fraction of the total ozone uptake to occur at night (Table 1).

The instantaneous individual flux threshold of $1.6 \text{ nmol m}^{-2} \text{ s}^{-1}$ proposed by Karlsson et al. (2004) to account for ozone detoxification at cellular level, was almost never exceeded at night, but it should be taken into account that at night the detoxification apparatus is likely to be less efficient (Musselman and Minnick, 2000). The experiments of Winner et al. (1989), for example, demonstrate a higher level of injury and reduced growth of *Brassica rapa* L. exposed to ozone at night with respect to plants exposed during the day, even though nighttime uptake was lower than daytime because $g_{s,n}$ was 75–80% lower than daytime G_s . Similarly, Matyssek et al. (1995) showed that also woody plants, *Betula pendula* L., were more effected by night-time ozone resulting in higher reduction in growth than those exposed to ozone in daylight, even if leaf injury was similar for both treatments.

In our case it is possible that a similar effect is taking place also in maquis species but it is also possible that in these species – already adapted to oxidative stress drought and light driven – the effect may be less evident. Unfortunately knowledge on night-time physiology and ozone effects on Mediterranean maquis species is still lacking and should be addressed. However our night-time ozone uptake represents a significant portion of the dose (Fig. 8; Table 1) and shouldn't be neglected in ozone risk assessment. It is also worth noticing that this “additional” dose partially counterbalances the dose “avoided” by Mediterranean plants as a consequence of stomatal closure during the typical Mediterranean summer drought.

Additionally, in other sites the night-time uptake may be even higher than ours, since the dunes orography determined particularly stable atmospheric conditions for the rear dune ecosystem at night – i.e. air masses were stratified –

which brought to low ozone concentrations in the lower atmospheric strata.

4 Conclusions

Sap flow technology allowed to determine the night-time water loss and $g_{s,n}$ response to environmental parameters of three Mediterranean shrub species, in conditions of high humidity and low wind speed where EC resulted to be heavily impaired. The night-time physiological response to environmental parameters of the three studied species was assessed and found to be consistent with the diurnal water saving strategy. The approach also allowed to determine night-time ozone stomatal uptake by these species, an additional dose 10 to 20% higher than the dose estimated by considering stomata to be closed at night. This additional dose could be particularly relevant for vegetation risk assessment (Fuhrer et al., 1997) especially if the higher toxicology of ozone at night will be proven. The night-time fraction may partially compensate the dose usually considered avoided by Mediterranean species as a consequence to the diurnal stomatal response to drought. Given the technological difficulties for the estimation of night-time fluxes, more research needs to be focused on this issue.

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